



Application No. 10/807,169
Attny Dkt No.: SUGI3001D/ESS

LISTING AND AMENDMENT OF THE CLAIMS:

1. – 9. (Canceled).

10. (Currently amended) A method of determining cAMP content or an adenylate cyclase activity in a biological sample comprising the following steps:

Cleaning Reaction: combining a biological sample with effective amounts of apyrase, alkaline phosphatase and adenosine deaminase to enzymatically remove endogenous non-cyclic adenine nucleotides other than cAMP, and endogenous ~~glucose-6-phosphagephosphate~~ glucose-6-phosphate;

Converting Reaction: enzymatically converting cAMP in the biological sample into ATP; and

Detecting Reaction: enzymatically converting ATP into fructose-6-phosphate which is then enzymatically converted into 6-phosphogluconolactone and NADPH and determining a concentration of NADPH without the use of radioactive agents.

11. (Original) The method according to Claim 10 further comprising, in said Cleaning Reaction, combining said biological sample with effective amounts of glucose oxidase, glycogen phosphorylase and alkaline phosphatase so as to enzymatically remove endogenous glycogen from said biological sample.

12. (Currently amended) The method according to Claim 10 wherein said Detecting Reaction comprises, after enzymatically converting fructose-6-phosphate into 6-phosphoglucono-lactone and NADPH, further ~~heatig~~ heating the reaction mixture and then adding 6-phosphogluconate dehydrogenase and NADP^+ so as to convert said 6-phosphogluconolactone into ribulose-5-phosphate and NADPH.

13 (Original) The method according to Claim 10 wherein in said Cleaning Reaction, endogenous non-cyclic adenine nucleotides other than cAMP is one or more of ATP, ADP. AMP and a mixture thereof.

14. (Previously presented) The method according to Claim 10 wherein, in said Converting Reaction, the conversion of cAMP into ATP is carried out by a combination of effective amounts of phosphodiesterase, myokinase and pyruvate kinase.

15. (Original) The method according to Claim 10 wherein, in said Detecting Reaction, the conversion of ATP into fructose-6-phosphate is carried out by a combination of hexokinase and pyruvate kinase.

16. (Original) The method according to Claim 10 wherein, in said Detecting Reaction, the conversion of fructose-6-phosphate into 6-phosphogluconolactone and NADPH is carried out by combination of phosphoglucose isomerase and glucose-6-phosphate dehydrogenase.

17. (Original) The method according to Claim 10 wherein, in said detecting reaction, an enzyme for conversion of ATP into fructose-6-phosphate is deactivated by a chelating agent after conversion of non-cyclic adenine nucleotides.

18. (Original) The method according to Claim 17 wherein said chelating agent is EDTA.

19. (Previously presented) The method according to Claim 10 wherein said biological sample is a mammalian tissue.

20. (Previously presented) The method according to Claim 10 wherein said biological is a physiological fluid.

21. (Currently amended) A kit for determining cAMP content or an adenylate cyclase activity in a biological sample which comprises:

(1) a vial for Cleaning Reaction comprising effective amounts of apyrase, alkaline phosphatase and adenosine deaminase to remove non-cyclic adenine nucleotides

consisting of endogenous ATP, ADP and AMP, and endogenous glucose-6-phosphate in a biological sample;

(2) a vial for Converting Reaction comprising an effective amount of phosphodiesterase to enzymatically convert cAMP in a biological sample into AMP; and

(3) a vial for Detecting Reaction comprising glycogen, inorganic phosphoric acid and glycogen phosphorylase to convert glycogen into glucose-1-phosphoric acid; phosphoglucomutase to convert glucose-1-phosphate into glucose-6-phosphate; and glucose-6-phosphate dehydrogenase and ~~NADP~~NADP⁺ to convert glucose-6-phosphate into 6-phosphogluconolactone and NADPH.

22. (Currently amended) A kit for determining cAMP content or adenylate cyclase activity in a biological sample which comprises:

(1) a vial for cleaning Reaction comprising effective amounts of apyrase, alkaline phosphatase and adenosine deaminase to enzymatically remove endogenous non-cyclic adenine nucleotides other than cAMP, and endogenous glucose-6-phosphate in a biological sample;

(2) a vial for Converting Reaction comprising effective amounts of phosphodiesterase, ATP, myokinase, phosphoenolpyruvic acid and pyruvate kinase to enzymatically convert cAMP in the biological sample into ATP; and

(3) a vial for Detecting Reaction comprising fructose and hexokinase to convert ATP into fructose-6-phosphate; phosphoglucose isomerase. glucose-6-phosphate dehydrogenase and ~~NADPT~~NADP⁺ to convert fructose-6-phosphate into 6-phosphogluconolactone and NADPH.

23. (Currently amended) The method according to Claim 11 wherein said Detecting Reaction comprises, after enzymatically converting fructose-6-phosphate into 6-phosphogluconolactone and NADPH, further heating the reaction mixture and then adding 6-phosphogluconate dehydrogenase and ~~NADP~~NADP⁺ so as to convert said 6-phosphogluconolactone into ribulose-5-phosphate and NADPH.